

# Assessment of Multiple Management Systems in the Upper Midwest

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## ABSTRACT

Independently, reduced tillage, multicrop rotations, and organic fertilization are expected to enhance soil biological properties; outcomes are less understood when these management strategies are integrated with conventional or organic system management. Over 9 yr, soil biological properties of microbial biomass C (MBC) and N (MBN) and soluble organic C (SolC) and total soluble N (SolN) were measured to evaluate these practices. A full factorial arrangement within each management system of the following strategies were used: 2-yr vs. 4-yr rotation; conventional tillage (CT) vs. strip tillage (ST); and unfertilized vs. fertilized with inorganic fertilizer or manure in the conventional and organic systems, respectively. Parsing out independent effects from integrated strategies demonstrated that across all years, MBC and MBN were not influenced by system management but were higher in 4-yr than 2-yr rotations, ST than CT, and fertilized than unfertilized treatments; SolC and SolN were greater in fertilized treatments and SolN greater in CT treatments. Independent strategies, however, reacted differently when integrated under the two management systems. Under conventional system management, MBC increased with time only when ST was combined with a 4-yr rotation, regardless of fertilization strategy; whereas, under organic system management, MBC increased with time in all ST treatments regardless of rotation or fertilization strategy. Results confirmed expectations that reduced tillage and increased rotation diversity positively impacted measured soil parameters, but inorganic vs. organic fertilizer resulted in few differences. Selecting and using individual strategies to achieve positive soil changes will depend on system-level management and how strategies are integrated.

MANAGEMENT PRACTICES THAT improve soil quality are essential to the maintenance of crop productivity (Doran and Zeiss, 2000). Soil organic matter (SOM) is frequently evaluated in agricultural production systems to determine the effects of different management practices. Increased SOM content and quality has been achieved by reducing tillage or increasing C inputs with manures, cover crops, or diverse cropping rotations, particularly those with legumes (Reicosky et al., 1995; Drinkwater et al., 1998; Ding et al., 2006). Changes in SOM content induced by specific management strategies may not be readily perceived (Sikora et al., 1996), however, particularly in soils already high in C content (>3%) such as agricultural soils converted from native prairie, as found in the Upper Midwest. In fact, 4 yr of implementation of a 4-yr rotation and strip tillage in these soils resulted in significant soil organic C increases in only the surface 0 to 5 cm (Johnson et al., 2011). Therefore, in this study, the focus was on soil biological parameters.

Soil biological parameters (i.e., MBC and MBN) are among the identified soil quality indicators (Andrews et al., 2004). Furthermore, biological parameters are linked to SOM processes, integrate many aspects of soil quality, and relate to soil ecosystem functioning. Microbial biomass increases have been linked to reduced tillage or increased C inputs (Alvarez et al., 1995, 1998; Karlen et al., 1994; Ritz et al., 1997; Peacock et al., 2001; Espershütz et al., 2007; Dinesh et al., 2009; Buyer et al., 2010; Nair and Ngouajio, 2012). Therefore, microbial biomass is a useful biological indicator because of its sensitivity to changes in quantity as well as quality of SOM and quick response to management changes (Gregorich et al., 1994).

Nitrogen cycling is a crucial aspect of the soil ecosystem function. Soil MBN is sensitive to soil N dynamics and organic matter mineralization, and changes in MBN may indicate potentially available N (Willson et al., 2001). For instance, high C/N ratio substrates can result in loss of microbial N, and low C/N ratio substrates can result in gains of microbial N (Paul and Clark, 1996, p. 131–146). Soil MBN has a variable relationship to MBC that can shift with management practices such as tillage. Conventional tillage may have lower MBC relative to MBN, indicating more bacteria, while no-till may have higher MBC relative to MBN, indicating more fungi (Balota et al., 2003). Therefore, MBN is likely to respond differently to management practices than MBC, and a better understanding of these differences is needed.

Dissolved organic C and N, comprised of soluble organic molecules <0.45  $\mu\text{m}$  in size, are important components of the

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**Abbreviations:** CT, conventional tillage; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SolC, soluble organic carbon; SolN, total soluble nitrogen; SOM, soil organic matter; ST, strip tillage.

SOM pool and influence both its size and turnover (Kalbitz et al., 2000; Neff and Asner, 2001). Dissolved organic C and N are sensitive to changes in agricultural management practices (Haynes, 2005). Dissolved organic C has been used as a measure of the labile C pool that is active in biological processes (Neff and Asner, 2001). Extractable active C has strong positive correlations with measures of microbial dynamics, especially MBC (Weil et al., 2003). Some forms of dissolved organic N can be used by plants for growth, although inorganic N is the typical indicator used for soil fertility management. Jones and Willett (2006) determined that dissolved organic C and N can be reliably measured concomitantly with microbial biomass and inorganic N extractions. A better understanding of the relationship of management factors to soluble C and N and microbial dynamics is needed.

In conventional production systems, reduced tillage or the use of cover crops is readily adopted because chemical weed control or cover crop termination is not compromised, while implementing diverse crop rotations might be deterred by initial infrastructure investments (Lyson and Welsh, 1993) and complex marketing strategies. In contrast, for organic production systems, reduced tillage can complicate the management of weeds or cover crops (Peigné et al., 2007), whereas inputs of organic and green manures, the use of legumes as N sources, and diverse rotations are common. Ample information is available on soil quality effects from individual management strategies, but less is known about their interactions for conventional systems. Conversely, less is known about individual strategies in organic systems because multiple strategies are usually used and can be critical for maintaining productivity and certification (Delate and Cambardella, 2004).

The objective of this study was to measure changes during a 9-yr period in MBC, MBN, SolC, and SolN within a large-scale comparison of conventional and organic system level management under which contrasting rotation, tillage, and fertility strategies were used. Microbial biomass C was expected to increase with a 4-yr crop rotation, strip tillage, and fertilizer amendments. Integration of these management strategies was expected to be synergistic, resulting in greater effects than using individual strategies alone. These patterns were expected under both conventional and organic system management; however, the use of an organic manure amendment was expected to increase MBC to a greater extent than an inorganic amendment. Soil MBN was expected to reflect the complexity of organic inputs from diverse rotations and reduced tillage. Measures of soluble C and N were included to evaluate management practices and associations with microbial biomass.

## MATERIALS AND METHODS

### Site Description

The study was conducted within a 3-ha area located on the Swan Lake Research Farm, near Morris, MN (45°41' N, 95°48' W), implementing a split-plot randomized complete block with four replications (Archer et al., 2007). A primary benefit of blocking is to minimize the contribution of known sources of variation among treatments (Gomez and Gomez, 1984). Soils in the region developed from glacial till and are variable across the study area; thus, blocking was used to reduce experimental error expected from known soil variability. Briefly, the five soil types occurring in

the study area were loams, clay loams, and silty clay loams ranging from 334 to 409 g kg<sup>-1</sup> sand and 227 to 271 g kg<sup>-1</sup> clay in the 0- to 30-cm depth. In the surface 0- to 15-cm depth, total soil C ranged from 32.4 to 36.6 g kg<sup>-1</sup>, total inorganic C ranged from 0.20 to 3.64 g kg<sup>-1</sup>, and pH ranged from 7.01 to 7.42 (for further details, see Johnson et al., 2011).

The experimental field was planted to soybean [*Glycine max* (L.) Merr.] and chisel plowed after the soybean harvest the year before plot establishment, which occurred in 2002. Experimental plots were 6 by 12 m, with 76-cm row spacing for corn (*Zea mays* L.) and soybean and 18-cm spacing for wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa* L.). Plots were established in four replicated blocks split into organic or conventional management systems, in which two contrasting rotation, tillage, and fertility treatment combinations were completely randomized in a full factorial arrangement; these combinations resulted in a total of 16 explicit integrated management strategies. The management system splits and replicate blocks were separated by a minimum of 12 m. Each of the four independent management strategies are described below; additional information can be found in Archer et al. (2007) and Johnson et al. (2010, 2011, 2012).

## Management Strategies

### System Management

The two systems evaluated were managed with conventional or organic management practices, with the main difference being the fertility source (inorganic fertilizers vs. organic manures) and weed management (chemical vs. mechanical means). For weed management in the conventional system, chemical pesticides appropriate for the target species, mainly glyphosate [*N*-(phosphonomethyl)glycine], were used. For weed management in the organic system, rotary hoe, in-row cultivation and interrow mowing were used in corn and soybean plots, and harrowing was used in wheat plots; planting was also delayed in some cases to allow further cultivation events. The timing of weed management was determined by using the Weed Cast Decision Aid (Archer et al., 2006; Oriade and Forcella, 1999). Although chemical insecticides were used under the conventional system management, organic insect pest management was not administered. Within both conventional and organic systems, two levels each of crop rotation, tillage, and fertility were established.

### Rotations

Crops were rotated in a traditional 2-yr corn–soybean or an alternative 4-yr corn–soybean–wheat/alfalfa–alfalfa rotation. Under both rotations, every crop was planted each year; therefore, each experimental plot had a specific invariable entry point. To meet organic certification requirements for the 2-yr rotation, a winter rye (*Secale cereale* L.) cover crop was hand broadcast under the corn crop midsummer through 2006 and then broadcast after the soybean harvest, to improve stand density, in early fall from 2007 to 2009. By 2007, the sixth growing season, weed pressure and difficulty rotating into corn in the organic system plots required eliminating the wheat nurse crop so that alfalfa could be managed more effectively.

Within both systems, the same or comparable crop cultivars were used from year to year with respect to maturity ratings;

organic cultivars were used when available. For conventional system corn, Pioneer 38W36 was used in 2002 and DK 4446 for all remaining years. For organic system corn, Pioneer 38W36 was used in 2002, DK 440 in 2003, NC 40M21 in 2004 and 2005, then Blue River 30-A12 for all remaining years. In conventional system soybean, Pioneer hybrids, mainly 91B33, were used. In organic system soybean, Hendricks (Clear hilum) was used in 2002, Pioneer 9091 the following year, then Vital for all remaining years. Alsen wheat was used in all plots in all years except 2005, when Oklee was used. Wrangler alfalfa was used in all plots all years excluding 2007, when Big N Annual was used in reconditioned second-year organic system alfalfa treatments.

### Tillage

Conventional tillage (CT) was conducted in fall with a moldboard plow, 0.25 m deep, following second-year alfalfa and corn, and a chisel plow, 0.25 m deep, following soybean; field cultivation, 0.12 m deep, was also used in corn, soybean, and wheat before planting. Strip tillage (ST) was conducted in fall with a 0.2-m-deep, 0.1-m-wide shank spacing centered on the row following corn or second-year alfalfa. Wheat underseeded with alfalfa was not tilled.

### Fertility

Two fertility treatments, fertilized or not fertilized, were established. In the conventional system, all fertilized corn and soybean received an N and P starter application at planting at 11 and 17 kg ha<sup>-1</sup>, respectively. In addition, all 2-yr corn received a broadcast or anhydrous NH<sub>3</sub> sidedress from 100 to 200 kg N ha<sup>-1</sup>, based on preplant soil test values using the ARS Nitrogen Decision Aid (Olness et al., 1999). In the conventional system 4-yr rotation, N, P, and K fertilizer was applied before planting wheat at 78, 15, and 28 kg ha<sup>-1</sup>, respectively. In the organic system, solid dairy manure was applied most years in the spring before planting only once per rotation: before corn in the 2-yr rotation and before wheat in the 4-yr rotation. Dairy manure moisture and thus dry matter content was highly variable, from 30 to 70% moisture, 6 to 52% total C, and 0.6 to 1% total N; applications, at 16,800 to 37,200 kg ha<sup>-1</sup>, ranged from 112 to 182 kg N ha<sup>-1</sup>, 15 to 29 kg P ha<sup>-1</sup>, and 105 to 170 kg K ha<sup>-1</sup>. Liquid swine manure (37,400 L ha<sup>-1</sup> with N, P, and K of 182, 25, and 170 kg ha<sup>-1</sup>, respectively) was applied once in place of the dairy manure, in fall of 2002 following soybean, instead of the following spring before 2-yr corn or 4-yr wheat. No organic fertilizer was applied in the 4-yr rotation in or after 2007 when wheat was eliminated from the rotation.

### Soil Sampling and Analysis

All 192 experimental plots were sampled in the spring of each year from 2002 through 2010 just after the yearly frost receded below a depth of 60 cm and the soil was firm enough to support foot traffic. Soil for soluble and microbial C and N was collected from the 0- to 15-cm depth at three locations along a centralized transect, avoiding surface residue and live plant roots (from alfalfa or winter rye), with a 2.5-cm-diameter manual soil probe and composited. Within hours of sampling, MBC and MBN were determined on sieved (0.45-cm), field-moist soil using the standard chloroform fumigation-direct

extraction technique (Vance et al., 1987). An additional subsample was dried to constant mass at 105°C for dry mass adjustments. Filtered extractions (Whatman no. 42, <2.5 µm) were stored frozen (-10°C) until analysis. Soluble organic C content was measured in both fumigated and unfumigated 0.5 mol L<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub> extractions by the chemical O<sub>2</sub> demand (COD) method for 2002 to 2006 and by direct combustion on a IL550 TOC/TN analyzer (Hach-Lange) for 2007 to 2010. Commercially prepared COD vials (CHEMetrics), containing Hg to eliminate Cl<sup>-</sup> interference, were used. Total soluble N content in fumigated and unfumigated extracts was measured for all years on the TOC/TN analyzer, with frozen archived extracts for 2002 to 2006 reanalyzed in 2007. The flush of microbial contents was determined as the difference between fumigated and unfumigated concentrations and converted into MBC and MBN by dividing by the following efficiency factors:  $k_{ec} = 0.38$  for MBC (Vance et al., 1987) and  $k_{en} = 0.45$  for MBN (Jenkinson et al., 2004). The analysis of archived extracts indicated a systematic error in the COD measurements taken in 2002, and these data were used only as a baseline to establish that no preexisting bias existed among treatments; 2002 data are not shown. Analysis of archived samples indicated that quantitative differences reached 25%; however, standards were comparable between methods (Weyers and Wilts, unpublished data, 2010). Therefore, the COD and TOC/TN analyses were combined to evaluate C dynamics across the 2003 to 2010 sample years. No original MBN data were available; therefore, we limited the statistical analysis of those data to the 2007 to 2010 sampling years; however, the data obtained from archived samples are provided for completeness.

Total soil C and inorganic C were measured in the fall after final harvest and before tillage in 2003 and 2009 at the 0- to 5-, 5- to 10-, and 10- to 15-cm depths following the methods outlined in Johnson et al. (2011). Briefly, total soil C was measured by dry combustion, inorganic C by an acidification pressurization test, and soil organic C calculated as the difference.

### Statistical Analyses

Soluble organic C, SolN, MBC, and MBN were analyzed using PROC GLIMMIX in SAS 9.2 software, with field block as the random variable and years treated as a repeated measure (SAS Institute, 2009). A clearly discernible trend with time indicated that effects from the prior year had an influence on the next; therefore, years could not be treated as random. Total soil organic C was analyzed only for comparison between 2003 and 2009 using PROC GLIMMIX, with field block as the random variable. Standard means and errors (SE) are presented. For all tests,  $\alpha = 0.05$ .

Because of the complexity of the data set, a degree-of-freedom conserving model statement was selected, whereby all interactions among management strategies (up to four-way), two-way interactions of each strategy with year, a three-way interaction between system, fertility, and year, and a single five-way interaction of all four strategies with year were evaluated (Table 1). Alternative model statements were evaluated and insignificant higher interactions with year that were not planned comparisons were dropped. The rotation strategy effect, with two levels, 2-yr and 4-yr, rather than an entry-point effect was chosen to simplify assessments; mixed model



**Table 1. Model effects and significance for microbial biomass C and N (MBC and MBN, respectively) and soluble C and N (SolC and SolN, respectively).**

Effect	MBC	MBN	SolC	SolN
System (Sys)	ns	ns	ns	ns
Rotation (Rot)	***	***	ns	ns
Tillage (Till)	***	***	ns	***
Fertility (Fert)	***	***	*	***
Sys × Rot	*	***	ns	***
Sys × Till	ns	*	ns	ns
Sys × Fert	ns	*	ns	ns
Rot × Till	*	ns	ns	ns
Rot × Fert	ns	ns	ns	ns
Till × Fert	ns	ns	ns	ns
Sys × Rot × Till	ns	ns	ns	ns
Sys × Rot × Fert	ns	ns	ns	ns
Sys × Till × Fert	ns	ns	ns	ns
Rot × Till × Fert	ns	ns	ns	ns
Sys × Till × Fert × Rot	ns	ns	ns	ns
Year	***	***	***	***
Sys × Year	***	ns	ns	ns
Rot × Year	*	ns	ns	ns
Till × Year	***	*	ns	ns
Fert × Year	*	ns	***	ns
Sys × Fert × Year	ns	ns	ns	ns
Sys × Till × Fert × Rot × Year	ns	ns	ns	ns

\* Significant at the  $P < 0.05$  level; ns, not significant.

\*\*\* Significant at the  $P < 0.001$  level.

procedures can manage an unbalanced design (Littell et al., 2006). Although crops changed within the plots from year to year, the analyses, which used plot as the repeated measures subject, precluded evaluation of specific crop effects.

The effects for the four independent management strategies were primarily defined from one-way main effects and two-way interactions with time. Two-way and higher level interactions among strategies were used to evaluate the effects of integrating strategies. The five-way interaction of the four strategies with time, as a planned comparison, was used to evaluate the explicit differences among the 16 integrated management strategies with time. The effect of fertilizer source, inorganic vs. manure, was evaluated with the two-way interaction of system and fertility and three-way interaction among system, fertility, and year. Multiple comparisons among treatment combinations were evaluated across or within years for planned comparisons and significant model effects. All  $P$  values were adjusted for multiplicity, particularly to control the familywise error rate for nonsignificant model effects on planned comparisons, using the Bonferroni method (Westfall et al., 2011). Comparisons among years for respective treatments were limited to 2003 vs. 2010 for MBC data and 2007 vs. 2010 for MBN data. To confirm change with time in MBC, general linear and quadratic regression analysis was conducted for each of the 16 system × rotation × tillage × fertility treatment combinations using yearly means over years in production using SAS software (SAS Institute, 2009).

## RESULTS

### Independent Strategy Comparisons

#### Microbial Biomass Carbon and Nitrogen

Parsed out from the integrated strategies, MBC and MBN averaged across all years of this study were different among rotation, tillage, and fertility treatments but not between the two management systems (Table 1). Three of the four management strategies had significant interactions with year for MBC. Within most years, multiple comparisons for these interactions indicated that 4-yr rotations were greater than 2-yr rotations, ST was greater than CT, and fertilized treatments were greater than unfertilized (Table 2). Despite the significance of main effects for MBN, only rotation and tillage exhibited a general consistency within years, whereas the fertilizer effect was limited to 2010. Limited differences within years were determined between systems, with MBC greater in 2010 and MBN greater in 2008 and 2010 in organic than conventional system management. Although increases in MBN paralleled increases in MBC, changes were not proportional but on average were 7% greater for system and tillage strategies, 4% less for fertility strategy, and 1% less for rotation strategy for the 2003 to 2010 period.

#### Soluble Carbon and Nitrogen

Averaged across all years, SolC and SolN were not influenced by system or rotation, both were influenced by fertilization strategy, but only SolN was influenced by tillage (Table 1). Using fertilizer increased both SolC and SolN, and CT increased SolN, but this overall effect was not consistent within years (Table 3). Comparisons within years for all strategies indicated that SolC was greater in the organic system in 2008 and 2010, greater under the 2-yr rotation in 2004, and greater in fertilized treatments in 2006, 2008, and 2010. Total SolN was greater in the conventional system in 2009, greater with CT in all but 2007, and greater in fertilized treatments in 2007 and 2009 (Table 3).

### Integrated Strategy Comparisons

#### Microbial Biomass Carbon

Only two, two-way interactions were significant for MBC (Table 1). The significant interaction between system and rotation indicated that the main effect of rotation was due entirely to the difference between the conventional system 2-yr rotation and the conventional system 4-yr rotation. No other comparisons were significant. For the conventional system 2-yr, conventional system 4-yr, organic system 2-yr, and organic system 4-yr rotations, values were 561, 634, 604, and 630 mg MBC kg<sup>-1</sup> soil (SE < 25), respectively. The rotation × tillage interaction, consistent with the main tillage effect, indicated that MBC was greater under ST than CT for both rotations; however, MBC was greater in 4-yr than 2-yr rotations only under ST. Thus 4-yr ST > 2-yr ST > (4-yr CT = 2-yr CT), which had values of 689, 614, 575, and 551 mg MBC kg<sup>-1</sup> soil (SE < 20), respectively.

The 16 integrated strategies displayed distinct trends for MBC with time, which were similar enough for paired fertilized and not fertilized strategies to be combined for simplicity (Fig. 1). These integrated strategies were compared in three ways: (i) regression analysis to describe trends with time, (ii) paired comparisons of starting and end points between years 2003 and 2010 for each of the 16 strategies, and (iii) multiple

**Table 2. Average microbial biomass C (MBC) and N (MBN), within and across years, reflecting the statistical differences of discrete management strategies including organic (ORG) or conventional (CNV) system, 2-yr (corn-soybean) or 4-yr (corn-soybean-wheat/alfalfa-alfalfa) rotation, strip tillage (ST) or conventional tillage (CT), and fertilized (YF) or not fertilized (NF).**

Year	System		Rotation		Tillage		Fertility	
	ORG	CNV	4-yr	2-yr	ST	CT	YF	NF
MBC, mg kg <sup>-1</sup> soil								
2003	496	497	499	492	499	495	518	476
2004	548	579	<b>579†</b>	<b>533</b>	<b>586</b>	<b>541</b>	574	553
2005	619	646	<b>650</b>	<b>599</b>	<b>666</b>	<b>599</b>	<b>662</b>	<b>603</b>
2006	618	617	<b>635</b>	<b>581</b>	<b>655</b>	<b>579</b>	625	610
2007	653	612	639	618	<b>688</b>	<b>577</b>	<b>661</b>	<b>604</b>
2008	675	663	<b>700</b>	<b>608</b>	<b>751</b>	<b>588</b>	<b>700</b>	<b>639</b>
2009	687	656	<b>695</b>	<b>623</b>	<b>736</b>	<b>607</b>	<b>700</b>	<b>642</b>
2010	<b>673</b>	<b>610</b>	<b>659</b>	<b>607</b>	<b>731</b>	<b>552</b>	<b>689</b>	<b>594</b>
Mean SE‡	16.91	15.27	14.00	19.17	16.33	13.87	16.34	15.59
Avg.	621	610	<b>632</b>	<b>583</b>	<b>664</b>	<b>567</b>	<b>641</b>	<b>590</b>
Avg. SE	6.39	5.73	5.32	7.02	6.46	5.09	6.19	5.81
MBN, mg kg <sup>-1</sup> soil§								
2003	91	97	97	89	100	89	93	96
2004	93	92	96	85	100	86	94	91
2005	110	110	114	103	118	103	113	108
2006	113	109	115	102	122	100	113	109
2007	115	105	111	106	<b>121</b>	<b>99</b>	114	105
2008	<b>154</b>	<b>130</b>	<b>146</b>	<b>133</b>	<b>166</b>	<b>118</b>	145	139
2009	124	115	<b>124</b>	<b>112</b>	<b>135</b>	<b>105</b>	123	116
2010	<b>112</b>	<b>96</b>	<b>108</b>	<b>97</b>	<b>120</b>	<b>88</b>	<b>110</b>	<b>99</b>
Mean SE	4.29	3.06	3.23	4.74	3.72	3.01	4.04	3.52
Avg.	126	111	<b>122</b>	<b>112</b>	<b>135</b>	<b>102</b>	<b>123</b>	<b>115</b>
Avg. SE	2.52	1.70	1.86	2.69	2.29	1.68	2.24	2.09

† Treatment comparisons in bold indicate a significant difference within year for adjusted  $P < 0.05$ .

‡ Mean SE is the mean of the standard errors determined within years, whereas average SE is for all data across all years.

§ 2003–2007 data for MBN are provided for completeness; however, they were not statistically analyzed. Mean SE, average, and average SE for MBN were determined only for 2007–2010.

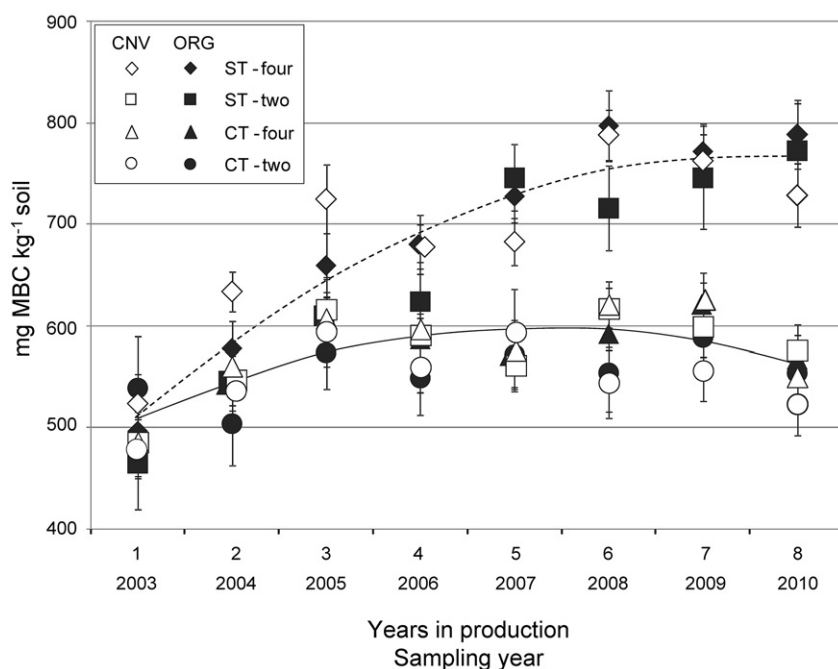
**Table 3. Average soluble organic C (SolC) and total soluble N (SolN), within and across years, reflecting the statistical differences of discrete management strategies including organic (ORG) or conventional (CNV) system, 2-yr (corn-soybean) or 4-yr (corn-soybean-wheat/alfalfa-alfalfa) rotation, strip tillage (ST) or conventional tillage (CT), and fertilized (YF) or not fertilized (NF).**

Year	System		Rotation		Tillage		Fertility	
	ORG	CNV	4-yr	2-yr	ST	CT	YF	NF
Organic SolC, mg kg <sup>-1</sup> soil								
2003	87	88	88	87	87	88	87	89
2004	80	81	<b>78†</b>	<b>86</b>	83	78	81	80
2005	61	62	60	64	62	61	60	63
2006	68	67	68	67	69	66	<b>71</b>	<b>64</b>
2007	59	57	57	61	59	57	61	55
2008	<b>64</b>	<b>58</b>	60	63	62	60	<b>63</b>	<b>59</b>
2009	45	42	43	44	44	42	45	41
2010	<b>66</b>	<b>60</b>	61	66	63	63	<b>66</b>	<b>59</b>
Mean SE‡	1.73	1.75	1.50	2.14	1.64	1.84	1.71	1.74
Avg.	66	64	64	67	66	64	<b>67</b>	<b>64</b>
Avg. SE	0.78	0.82	0.69	0.98	0.78	0.82	0.77	0.83
Total SolN, mg kg <sup>-1</sup> soil§								
2003	17.7	17.4	16.6	19.5	16.2	18.9	19.4	15.8
2004	12.2	13.0	12.4	13.2	11.7	13.5	13	12.5
2005	6.5	6.6	6.6	6.3	6.3	6.8	6.7	6.3
2006	7.3	9.4	8.7	7.6	7.9	8.8	8.6	8.0
2007	8.9	9.5	9.3	9.2	9.2	9.3	<b>10.1</b>	<b>8.4</b>
2008	13.6	13.3	13	14.0	<b>12.0</b>	<b>14.8</b>	13.5	13.3
2009	<b>8.5</b>	<b>9.5</b>	8.8	9.3	<b>8.4</b>	<b>9.5</b>	<b>10.0</b>	<b>8.0</b>
2010	11.1	10.8	10.9	11.0	<b>9.7</b>	<b>12.2</b>	11.6	10.3
Mean SE	0.47	0.51	0.44	0.54	0.43	0.52	0.49	0.46
Avg.	10.5	10.8	10.5	10.9	<b>9.8</b>	<b>11.4</b>	<b>11.3</b>	<b>10.0</b>
Avg. SE	0.26	0.27	0.24	0.30	0.23	0.29	0.26	0.27

† Treatment comparisons in bold indicate a significant difference within year for adjusted  $P < 0.05$ .

‡ Mean SE is the mean of the standard errors determined within years, whereas average SE is for all data across all years.

§ 2003–2006 data for total SolN are provided for completeness; however, they were not statistically analyzed. Mean SE, average, and average SE for SolN were determined only for 2007–2010.



**Fig. 1. Microbial biomass C (MBC) by growing season, showing years in production and sampling year, for eight integrated strategies under conventional (CNV) and organic (ORG) system management, averaged across fertilized and unfertilized treatments, where strip tillage (ST) or conventional tillage (CT) and 2-yr or 4-yr crop rotations were used. Production strategies separated into two groups, one with a significant increase with time,  $MBC = -6.2t^2 + 93t + 417$ ,  $r^2 = 0.86$ , and one without,  $MBC = -4.9t^2 + 52t + 455$ ,  $r^2 = 0.56$ , where  $t$  is years in production. Bars indicate 1 SE.**

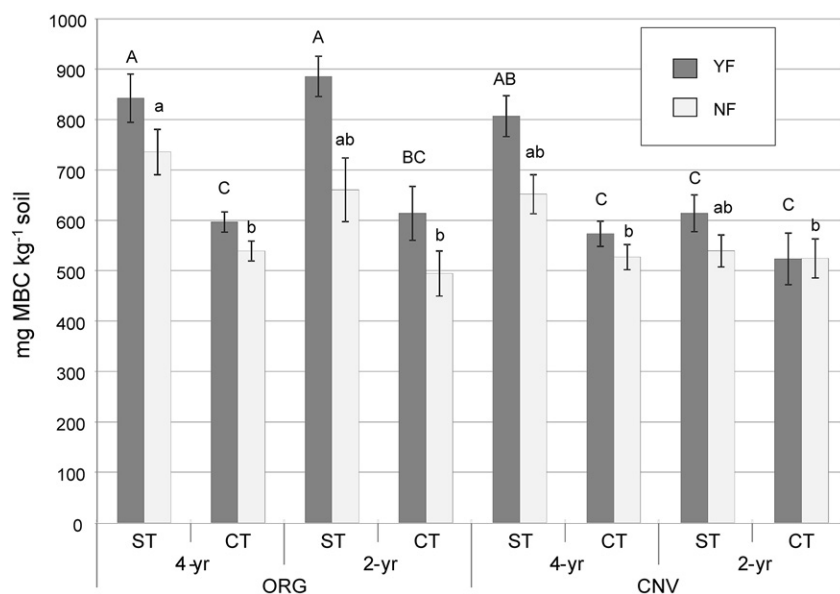
comparisons among the 16 strategies partitioned into fertilized or not fertilized treatments at the 2010 endpoint. Quadratic regressions on yearly MBC means separated the strategies into two groups. Three of the integrated strategies, combined across fertility treatments, were in the top group, which consisted of the organic system 2-yr and 4-yr rotations with ST and the conventional system 4-yr rotations with ST (shown as ORG 2-yr ST, ORG 4-yr ST, and CNV 4-yr ST, respectively, in Fig. 1). The lower group consisted of the remaining five strategies combined across fertility treatments, which were the four conventional and organic system 2-yr and 4-yr rotations with CT and the one conventional system 2-yr rotation with ST (shown as CNV 2-yr CT, CNV 4-yr CT, ORG 2-yr CT, ORG 4-yr CT, and CNV 2-yr ST, respectively, in Fig. 1). Regression slopes for each top group strategy were significantly different from zero ( $P < 0.001$ ), confirming that MBC accrued with time. Although slopes were not different from zero for the bottom group strategies, quadratic equations rather than linear had better fit for some, indicating a potential but not definite decline in MBC. Paired comparisons confirmed that MBC was greater in 2010 than 2003 for both fertilized and not fertilized integrated strategies in the top group but were not different between these years among the other 10 integrated strategies. Multiple comparisons at the 2010 endpoint among the eight fertilized strategies confirmed that MBC was similar within the two groups and different between them; this pattern was not as distinct for the unfertilized treatments (Fig. 2).

### Microbial Biomass Nitrogen

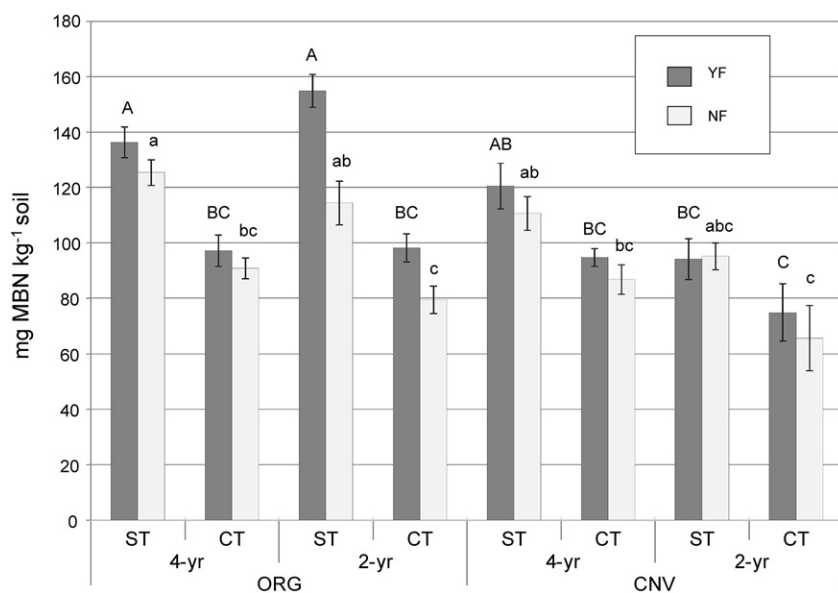
Three two-way interactions were significant for MBN (Table 1). The system and rotation interaction indicated that MBN was significantly lower in the conventional system 2-yr rotation than the conventional system 4-yr, organic system 2-yr, and

organic system 4-yr rotations collectively, with values of 96, 111, 112, and 116 mg MBN kg<sup>-1</sup> soil ( $SE < 3.0$ ), respectively. The system  $\times$  tillage interaction was not entirely consistent with the main tillage effect of greater MBN under ST than CT. Although MBN was greater under ST than CT within the management systems, the conventional system with ST was similar to the organic system with CT. For organic ST, conventional ST, organic CT, and conventional CT, MBN showed values of 128, 117, 100, and 97 mg kg<sup>-1</sup> soil ( $SE \leq 2.0$ ), respectively. Multiple comparisons for the system  $\times$  fertility interaction indicated that MBN was significantly greater in the organic fertilized treatment than all other treatments. The organic fertilized, conventional fertilized, organic not fertilized, and conventional not fertilized treatments showed values of 133, 120, 113, and 110 mg MBN kg<sup>-1</sup> soil ( $SE < 4.0$ ), respectively. These interactions reflect the complexity of system management effects compared with more clear-cut expectations for rotation, tillage, and fertility strategies.

Trends for MBN measurements for the 16 integrated strategies were not as distinct as those for MBC (Fig. 1), perhaps because the analysis of these data was limited to the last 4 yr of the study. Neither regression slopes nor paired comparisons between 2007 and 2010 indicated differences with time (data not shown). This lack of change with time in MBN demonstrated an inconsistent relationship with MBC, despite the relationship of MBC with MBN appearing to be fairly constant, averaging 6:1. Multiple comparisons for MBN within 2010, however, did confirm somewhat similar groupings for the 16 integrated strategies as found for MBC (Fig. 3). For fertilized strategies, MBN in the two organic system 2-yr and 4-yr rotation strategies with ST was different from MBN in all four strategies with CT as well as the conventional system 2-yr rotation with ST; however, MBN in the conventional



**Fig. 2. Microbial biomass C (MBC) for the 2010 sampling occurring after 8 full yr in production for the 16 integrated rotation, tillage, and fertility strategies under conventional (CNV) and organic (ORG) management systems with 2-yr or 4-yr crop rotations, under strip tillage (ST) or conventional tillage (CT) and fertilized (YF) and not fertilized (NF) treatments. Letters indicate significant differences ( $P < 0.05$ ) within YF treatments (uppercase) and NF treatments (lowercase). Bars indicate 1 SE.**



**Fig. 3. Microbial biomass N (MBN) for the 2010 sampling occurring after 8 full yr in production for the 16 integrated rotation, tillage, and fertility strategies under conventional (CNV) and organic (ORG) management systems with 2-yr or 4-yr crop rotations under strip tillage (ST) or conventional tillage (CT) and fertilized (YF) and not fertilized (NF) treatments. Letters indicate significant differences ( $P < 0.05$ ) within YF treatments (uppercase) and NF treatments (lowercase). Bars indicate 1 SE.**

system 4-yr ST fertilized strategy, which was in the top distinguished group for MBC, was different only from MBN in the conventional system 2-yr CT fertilized strategy. The MBN in the unfertilized strategies had a similar trend but not similar statistical differences.

### Soluble Carbon and Nitrogen

Interactions were not significant for SolC measurements but were for SolN (Table 1). The system  $\times$  rotation interaction indicated that the main effect of rotation on SolN was due primarily to significantly higher measurement in the conventional system

2-yr rotation, the opposite of that found for MBC and MBN. Total SolN was similar between the conventional system 4-yr rotation and each of the organic 2-yr and organic 4-yr rotations but was significantly greater in the organic 4-yr than the organic 2-yr rotation. For the conventional 2-yr, conventional 4-yr, organic 2-yr, and organic 4-yr rotations, these values were 12.0, 10.1, 9.7, and 10.9 mg SolN kg<sup>-1</sup> soil (SE < 0.5), respectively. The contrasting differences with systems explain why no main rotation effect for SolN occurred. The 16 integrated strategies exhibited no distinct separation patterns with time for SolC or SolN (data not shown). Averaged across all strategies, there was

a decline from 88 to 63 mg SolC kg<sup>-1</sup> soil from 2003 to 2010, respectively. Across all strategies and all four evaluation years, SolN averaged 11 mg kg<sup>-1</sup> soil.

### Total Soil Organic Carbon

Total soil organic C did not change from 2003 to 2009 for most integrated treatments (data not shown). The only significant gains with time were observed in the 0- to 5-cm depth of two integrated systems: the organic system, 2-yr rotation with ST and fertilizer, with a 23% gain, and the conventional system, 4-yr rotation with ST and fertilizer, with a 10% gain. Loss of total soil organic C was significant in only three integrated systems: the organic system, 4-yr rotation with CT and fertilizer, with an 8% loss in the 5- to 10-cm depth and a 6% loss at the 10- to 15-cm depth, and the fertilized and unfertilized conventional system, 2-yr rotations with CT, both with an 11% loss at the 5- to 10-cm depth. These minimal differences were not further evaluated.

### Fertilizer Source Effect

The overall fertilizer source effect, system × fertility × year interaction, was not significant with time for any parameter measured but was for the system × fertility two-way interaction for MBN, as discussed above (Table 1). This significant two-way interaction did indicate that the use of manure potentially increased the retention of N by the microbial biomass; however, planned comparisons to evaluate within-year effects indicated that MBN was greater in the organically managed fertilized treatment than the conventionally managed fertilized treatment only in 2008, with values of 155 and 124 g MBN kg<sup>-1</sup> soil (SE < 10), respectively. Organically managed fertilized treatments had no differences in MBC, SolC, or SolN than conventionally managed fertilized treatments in any year (data not shown).

## DISCUSSION

### Independent Management Strategy Effects

#### System Management

System-level management strategy differences between conventional and organic for any measured parameter did not become significant before six full years of production (2008). These results are in stark contrast to other studies in which the improvement of soil quality, mainly as increased microbial biomass, was more immediate in transitioning systems and continued to persist with long-term establishment of organic practices. These findings may relate to the soil type and initial SOM content. For example, Tu et al. (2006) established differences in both MBC and MBN within 3 yr of organic transition on Ultisols and Entisols with organic matter contents <8.4 g kg<sup>-1</sup> soil. Differences in MBC and MBN occurred within 2 yr of transition and persisted for at least 5 yr in an organic system on an Entisol, presumably starting at 1.2 g organic C kg<sup>-1</sup> soil (Araújo et al., 2008; Santos et al., 2012). Bending et al. (2000) showed sensitivity of microbial biomass and labile organic matter indicators to management level changes after a 2-yr transition to organic management on Inceptisols (Wick series by the UK classification) with 8.6 g organic C kg<sup>-1</sup> soil. In Mollisols ranging from 15 to 20 g organic C kg<sup>-1</sup> soil, four of five organic production systems, compared with conventional counterparts, had greater to significantly greater MBC and MBN when

evaluated 10 to 30 yr after establishment (Liebig and Doran, 1999). In light of these studies, the current findings obtained in Mollisols with organic C contents >33 g kg<sup>-1</sup> soil support the assessment that quality changes in soils high in organic matter may be difficult to perceive for system-level management changes applied for <6 yr.

### Rotation

Greater diversity and inputs of C sources feeding the SOM pool, particularly from crop rotations including legumes, enhance the diversity and resilience of soil biota (Altieri, 1999), which can enhance nutrient cycling and crop productivity. In confirmation of this view, the present study showed a significant positive impact on MBC and MBN in the more diverse 4-yr rotation than the 2-yr rotation. Data on harvested, unharvested, and root biomass in these cropping systems (Johnson et al., 2011) indicated that residue quality and root production were contributing factors for these observations. The 30% greater alfalfa root production compared with corn (Johnson et al., 2011, 2012), as well as its recalcitrance (Johnson et al., 2007), are supportive of a legume effect. Similar findings of greater MBC and MBN in diverse alternative systems incorporating various legumes like alfalfa and lentil (*Lens culinaris* Medik.) were found across multiple locations in the Great Plains (Liebig et al., 2006).

According to a review by Chantigny (2003), greater root exudation from a legume crop in a rotation could increase water-extractable organic C. Although the alfalfa crop with its perennial root system may have given rise to more SolC, there was minimal difference between the 4-yr and 2-yr rotations. The greater MBC under the 4-yr rotation suggests that this larger microbial community might have immobilized the labile C by using it as a substrate for growth. Studies with <sup>13</sup>C labeling of roots and residues support the idea that soil microbes might incorporate more C from plant roots than aboveground residue into their biomass (Kong et al., 2011).

### Tillage

Tillage was the most pronounced and first manifested effect of a specific management strategy on soil quality parameters. Separation between CT and ST confirmed that reducing soil disturbance by tilling only in strips resulted in the accretion of MBC and MBN with time, with proportionally more N immobilized in the biomass under ST. Increased microbial biomass probably was linked to an increase in organic matter resources in the surface 0 to 15 cm, although this was not directly attributable to any surface residue accumulation. The increase in MBN was not indicative of any positive or negative response of the bacterial or fungal component of the biomass to changes in tillage strategy. Increased SolN in CT over ST treatments was probably due to greater overall tillage disturbance, which increases mineralization, particularly nitrification (Calderón et al., 2001; Jackson et al., 2003), although the opposite effect, depending on soil depth, has also been noted (Cookson et al., 2008). Tillage effects on the soil biota can cause changes in soil function (Kladivko, 2001; Cookson et al., 2008), which could lead to short-term increases in mineralization due to increased porosity but long-term reductions in mineralization processes due to decreased resources and soil microbial biomass.



## Fertilization

Fertilized treatments showed increased MBC, with a tendency towards higher MBN, due to manure use over inorganic fertilizer. Manure additions might increase the microbial biomass because of additional C inputs (Fließbach et al., 2007; Nair and Ngouajio, 2012) or improvements in soil structure (Haynes and Naidu, 1998; Bronick and Lal, 2005). Manure applications introduce microbes that alter soil community assemblages (Bucher and Lanyon, 2005; Carrera et al., 2007), but these studies did not indicate if the introduced microbes increased the total soil biomass. Manure applied may have varied in quality but at maximum application approximated  $91 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , similar to the  $93$  to  $99 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  reported by Mäder et al. (2002) for a long-term organic and biodynamic conversion study in Switzerland but far below the  $375$  to  $750 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  estimated by Nair and Ngouajio (2012). Not finding a consistent influence of manure N vs. inorganic N within years on microbial biomass in the present study was due to the following possible factors: (i) manure condition (i.e., stabilized or unstabilized); (ii) the shorter term analysis of 9 yr in this study compared with the 21-yr assessment of the study in Switzerland (Fließbach et al., 2007); (iii) the infrequency and reduced rate of manure applications compared with Nair and Ngouajio (2012); or (iv) timing our analysis to just the spring rather than after manure application or throughout the growing season as in Ritz et al. (1997). In all cases, the end result was probably the equal contribution of organic matter inputs between the manure and inorganic fertilizer systems.

As reviewed by Chantigny (2003), long-term organic or inorganic fertility treatments can induce microbial processing and/or solubilization of organic matter; although the process in conventional systems is generally short term, organically managed soils exhibit longer term effects. The concurrent increases in both SolC and SolN with greater MBC and MBN with time in these spring-sampled soils support the interpretation of increased microbial processing over solubilization of existing organic matter or extraction of soluble manure components.

## Integrated Management Effects

Anticipated system-level main effects were apparent only with specific rotation, tillage, and fertility combinations. In the conventional system, strip tillage combined with the more diverse 4-yr rotation increased MBC to an extent greater than either strategy alone (i.e., conventional or strip tillage with a 2-yr rotation), indicating a synergistic effect. In the organic system, synergy with other strategies was absent because MBC increased only where strip tillage was used. This observation suggests that reduced tillage, rather than crop diversity or manure additions, caused gains in SOM, although not statistically measureable, particularly for organic system treatments; however, the winter cover crop used in the 2-yr rotation to maintain organic certification standards may have contributed to the positive effect on MBC. Weed biomass production was also unaccounted for in the organic system. Although weeds were managed with cultivation, harrowing, mowing, and hand pulling, C inputs from weed production were possible.

Soluble organic C (particles  $<2.5 \mu\text{m}$ ) was used as an analogue for dissolved organic C (particles  $<0.45 \mu\text{m}$ ), which is considered a good indicator of soil quality through its linkage

to total SOM and microbial biomass. Weil et al. (2003) determined that active soil organic C had a significant and strong positive correlation with MBC, as well as significant but weak correlations with total organic C and SolC (as measured in this study). In the present study, a negative linkage was found as MBC increased with time for some integrated strategies, while SolC apparently declined with time. Within years, however, independent comparisons of system, tillage, and fertility management strategies showed an apparent positive linkage where higher MBC was matched with higher SolC. The higher within-year SolC could indicate an increase in dissolved organic matter from manure applications or greater residue inputs (Chantigny, 2003). This assumption accommodates the functional linkage between dissolved organic C and microbial biomass suggested by Neff and Asner (2001) in that the increased microbial biomass processes more of this added organic matter and releases more soluble and thus measurable organic C. With time this could result in the increased microbial immobilization of the available C that was observed.

Total SolN dynamics corresponded best with management strategies that increase mineral N availability for crop growth, which were CT and 2-yr crop rotations using more frequent N amendments, and corresponded least with strategies that would immobilize N, in this case ST and unfertilized treatments. The SolN dynamics matched with greater yields in the conventional system 2-yr rotation CT treatments and lower yields in the organic system 2-yr rotations and all unfertilized treatments, as observed by Archer et al. (2007) and Johnson et al. (2011, 2012). The increase in SolN in CT treatments might indicate increased mobility and potential loss of available N before plant uptake.

The occurrence of low SolN with high MBN, particularly in organic and ST treatments, was indicative of microbial immobilization of available N. This immobilization was consistent with increased availability of C, indicated by SolC. Burger and Jackson (2003) attributed immobilization of  $\text{NO}_3$  to the greater inputs and availability of C in an organic system compared with an inorganically fertilized conventional system. In contrast, the decoupling of N cycling from soil C availability, observed in fertilized agricultural production systems (Booth et al., 2005), was evidenced by the greater SolN and lower microbial biomass, particularly in the conventional system CT treatments. These findings suggest the robustness of the microbial community to adapt to, maybe even control, N availability, at least in the short term (Kushwaha et al., 2000; Willson et al., 2001). Further, these observations support reasoning that management systems promoting soil quality have tighter nutrient cycling through strong coupling of N and C availability, resulting in N retention and long-term stabilization by the soil microbial biomass (Matson et al., 1997; Altieri, 1999).

The present study demonstrated that the use of CT imparted no positive changes in the soil parameters measured under either conventional or organic system management. Extensive tillage management might compromise the building of SOM, negatively affecting soil function by harming the soil biota and internal cycling and efficient use of nutrients (Matson et al., 1997). In addition, the inability to reduce tillage and adequately control weeds could compromise an organic production system's capacity to achieve and maintain better soil quality (Teasdale et al., 2007). Greater addition of organic

amendments, however, might compensate for CT (Liebig and Doran, 1999). In light of these results, attempting some of the management strategies such as reduced tillage, more diverse rotations, and organic amendments before making an organic management transition might prepare the land for the absence of conventional amendments (e.g., inorganic fertilizers, herbicides, insecticides, etc.), and protect the producer from yield-reduction losses in the absence of compensatory organic premiums during transition years (Archer et al., 2007).

## CONCLUSIONS

The study performed here showed that reducing tillage and increasing crop rotation diversity resulted in soil improvement, particularly from increased MBC. The effect of system management on the soil microbial biomass was affected by the strategies used in that management practice, requiring integrated strategies under a conventional systems approach but only one strategy, reduced tillage, under an organic systems approach. Positive impacts were noted in unfertilized treatments, indicating that rotation and tillage have a more profound influence on soil properties. Although positive aspects of organic fertilizer, in the form of manure, were not discounted, conventional fertilizer use in combination with conservation practices was also effective. Maintaining the sustainability of agricultural production systems depends on the use and integration of management strategies that provide sufficient organic matter resources to support soil biota and internal cycling and efficient use of nutrients.

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